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L2: Entry 15 of 46 File: USPT Jun 26, 2001

DOCUMENT-IDENTIFIER: US 6251588 B1
TITLE: Method for evaluating oligonucleotide probe sequences

BSPR:
Direct detection of labeled target hybridized to surface-bound probes is particularly advantageous if the surface contains a mosaic of different probes that are individually localized to discrete, known areas of the surface. Such ordered arrays containing a large number of oligonucleotide probes have been developed as tools for high throughput analyses of genotype and gene expression. Oligonucleotides synthesized on a solid support recognize uniquely complementary nucleic acids by hybridization, and arrays can be designed to define specific target sequences, analyze gene expression patterns or identify specific allelic variations. One difficulty in the design of oligonucleotide arrays is that oligonucleotides targeted to different regions of the same gene can show large differences in hybridization efficiency, presumably due, at least in part, to the interplay between the secondary structures of the oligonucleotides and their targets and the stability of the final probe/target hybridization product. A method for predicting which oligonucleotides will show detectable hybridization would substantially decrease the number of iterations required for optimal array design and would be particularly useful when the total number of oligonucleotide probes on the array is limited. A method to predict oligonucleotide hybridization efficiency would also streamline the empirical approaches currently used to select potential antisense therapeutics, which are designed to modulate gene expression in vivo by hybridizing to specific messenger RNA (mRNA) molecules and inhibiting their translation into proteins.

BSPR:
Unfortunately, these conditions do not apply to oligonucleotide arrays, which are usually hybridized under relatively non-denaturing conditions, or to antisense suppression of gene expression, which takes place in vivo. Oligonucleotide arrays can contain hundreds of thousands of different sequences and conditions are chosen to allow the oligonucleotide with the lowest melting temperature to hybridize efficiently. These "lowest common denominator" conditions are usually relatively non-denaturing and secondary structure constraints become significant. Accordingly, the above applications require new predictive methods that are capable of estimating the effects of oligonucleotide and target structure on hybridization efficiency. For these reasons, current algorithms for designing PCR primer oligonucleotides fail badly when applied to the problems of oligonucleotide array or antisense oligonucleotide design.

L2: Entry 4 of 46 File: USPT Dec 25, 2001

DOCUMENT-IDENTIFIER: US 6333155 B1
TITLE: Exploiting genomics in the search for new drugs

DEPR:
Normalization controls are oligonucleotide or other nucleic acid probes that are complementary to labeled reference oligonucleotides or other nucleic acid sequences that are added to the nucleic acid sample. The signals obtained from the normalization controls after hybridization provide a control for variations in hybridization conditions, label intensity, "reading" efficiency and other factors that may cause the signal of a perfect hybridization to vary between arrays. In a preferred embodiment, signals (e.g., fluorescence intensity) read from all other probes in the array are divided by the signal (e.g., fluorescence intensity) from the control probes thereby normalizing the measurements.

DEPR:
Virtually any probe may serve as a normalization control. However, it is recognized that hybridization efficiency varies with base composition and probe length Preferred normalization probes are selected to reflect the average length of the other probes present in the array, however, they can be selected to cover a range of lengths. The normalization control(s) can also be selected to reflect the (average) base composition of the other probes in the array, however in a preferred embodiment, only one or a few normalization probes are used and they are selected such that they hybridize well (i.e. no secondary structure) and do not match any target-specific probes.

DEPR:
However, there may exist 20-mer subsequences that are not unique to the IL-2 mRNA Probes directed to these subsequences are expected to cross-hybridize with occurrences of their complementary sequence in other regions of the sample genome. Similarly, other probes simply may not hybridize effectively under the hybridization conditions (e.g., due to secondary structure, or interactions with the substrate or other probes). Thus, in a preferred embodiment, the probes that show such poor specificity or hybridization efficiency are identified and may not be included either in the high density array itself (e.g., during fabrication of the array) or in the post-hybridization data analysis.